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THE UREA CLEARANCE AS A MEASURE OF RENAL FUNCTION*

By DONALD D. VAN SLYKE

The Hospital of the Rockefeller Institute for Medical Research, New York City, N. Y.

Definition and Significance of the Urea Clearance[1]

The average normal adult, when excreting urine in fairly abundant volume, excretes per minute the amount of urea contained in 75 cc. of his blood. The excretion suffices to clear the urea from this volume of his blood. Hence we use the term "urea clearance" to indicate *the volume of blood which one minute's excretion clears of urea.*

The same volume of blood is cleared, regardless of whether its urea content at the moment is high or low. If, by feeding urea, the blood content is greatly increased, the excretion rate increases proportionately, so that the amount of urea excreted per minute remains equal to the amount contained in 75 cc. of blood.

"Clearance" serves as a convenient term to picture the direct relation between blood urea content and rate of excretion. A clearance of 75 does not indicate that 75 cc. of blood flow through the kidneys per minute, with complete removal of the urea. On the

[1] An outline of the physiological work of Marshall, Addis and others from which the clearance evolved may be found in the chapter on urea in Quantitative Clinical Chemistry, Volume I, of Peters and Van Slyke.

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contrary, to judge from experiments carried out by Rhoads, Hiller, Alving and the writer (1934), about ten times as great a volume of blood perfuses the kidneys, and only one-tenth of its urea is excreted.

These experimenters furthermore found that in dogs the urea clearance varied in direct proportion to variations in blood flow. It is probable that in man the same relationship holds under most normal and pathological conditions, and that a low urea clearance usually indicates a correspondingly low rate of renal blood flow.

Clearance Equations

The routine calculation of the clearance is done graphically, as will be detailed later, merely by laying a straight-edge across the scales of a line-chart. The equations on which the calculation is based will be given here, however, as they serve to show clearly the quantitative relation that is measured between blood urea concentration and rate of urea excretion.[2]

The following symbols are used:

B is urea concentration in blood.

U is urea concentration in urine.

V is the volume of urine in cc. excreted per minute.

$\%\ C_{Ur}$ is the urea clearance, expressed in per cent of the average normal value.

The clearance equations are developed from the following considerations. The ratio U / B indicates the number of cc. of blood, the urea content of which is compressed into 1 cc. of urine. Then $V \times U / B$ indicates the cc. of blood, the urea content of which is contained in the urine excreted in 1 minute. In other words, $V \times U / B$ is the actual urea clearance in cc. of blood cleared per minute.

Since with urine flows exceeding 2 cc. per minute the average normal clearance is 75 cc. of blood per minute, we multiply any observed actual clearance by 100/75, or 1.33, in order to calculate its percentage of average normal. Hence we obtain Equation 1.

(1) $\%\ C_{Ur} = \dfrac{1.33\ U\ V}{B}$, when V exceeds 2 cc. per minute.

When V falls below 2 cc. of urine per minute the clearance, in cc. of blood cleared, is no longer independent of V, but begins to decrease as the urine volume falls. To correct for this effect,

[2] A more complete development of the clearance formulae is given in the urea chapter of Peters and Van Slyke.

Equation 2 is used when the urine volume is in this low range.

(2) $\%\ C_{Ur} = \frac{1.85\ U\ \sqrt{V}}{B}$, when V is less than 2 cc. per minute.

The necessity for using a different equation when the urine output is below a certain volume was shown by Austin, Stillman and Van Slyke (1921), and Moller, McIntosh and Van Slyke (1928), who found that when V dropped below what they termed the "augmentation limit," which was about 2 cc., the volume of blood cleared of urea per minute, viz., the clearance calculated as $V \times U/B$, began to diminish, and that the diminution was parallel to the square root of V. This effect is shown by Fig. 1. Also, when V was 1, so that $U\ B \times \sqrt{V} = U\ B \times V$, it was found that the average normal cc. of blood cleared per minute was 54. Hence, to calculate the clearance in percentage of average normal by the square root formula, the factor used in Equation 2 is $100/54 = 1.85$.

In applying the equations V must always be expressed as cc. of urine per minute in order to obtain clearance values numerically comparable with those heretofore published. U and B, however, since they occur only in the ratio U/B in both equations, can be expressed in any convenient concentration units so long as both U and B are expressed in the *same* unit. They may both be calculated in grams per 100 cc., or both in mg. per 100 cc., and both as urea substance or both as urea nitrogen. But if B is calculated as mg. urea nitrogen per 100 cc. blood, U must be calculated as mg. urea nitrogen per 100 cc. urine. These are the units routinely used in the writer's laboratory.

Urea Clearance Calculated from Excretion of Ammonia plus Urea Nitrogen

The ammonia nitrogen of the urine averages about 5 per cent of the urea nitrogen. Hence if one were to calculate U as urea + ammonia nitrogen, instead of urea nitrogen alone, the effect would be to raise the average clearance value by about 1/20. The change would not usually be of importance in clinical interpretation.

However, when acidosis increases the ammonia excretion, or low nitrogen metabolism decreases the urea output, the ammonia nitrogen may amount to much more than 1/20 the urea. Under such conditions recent observations (Van Slyke, Page, Hiller and Kirk [1935]) have shown that the clearance when calculated by taking urea + ammonia nitrogen of the urine as U, is actually more consistent than when calculated with the exact urea nitrogen concen-

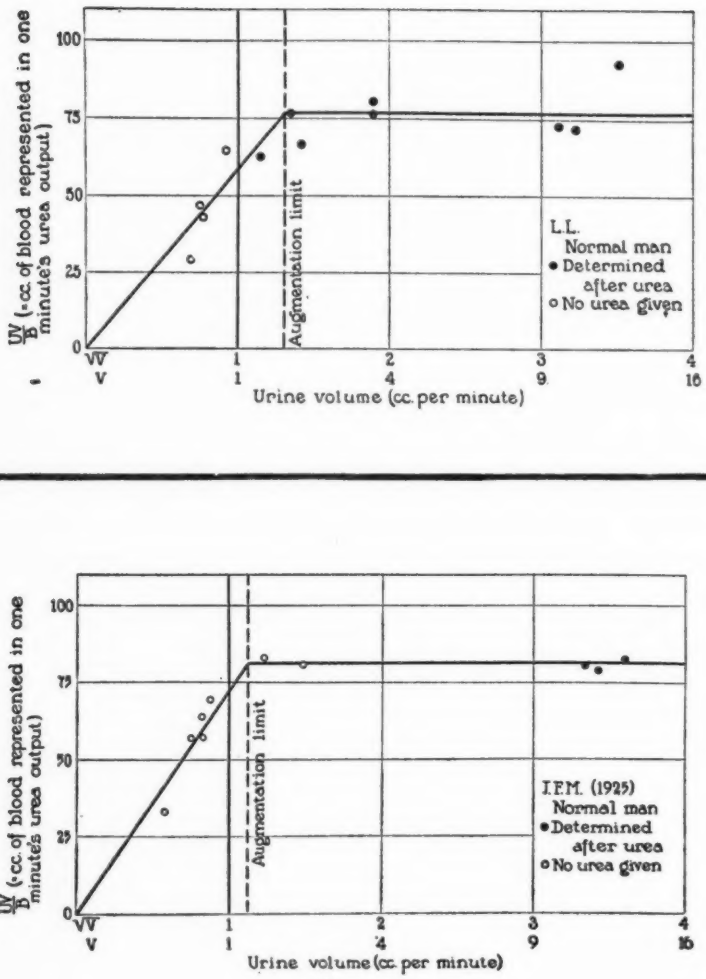

Figure 1

Fig. 1. The effect of urine volume changes on the value of the actual clearance in two normal men. Above the augmentation limit the clearance does not change with rate of urine excretion. Below the augmentation limit the clearance decreases at a linear rate with the square root of the urine volume.

tration as U. For example, a subject with normal renal function was put on a low protein diet to diminish the denominator of the NH_3/Urea ratio, and was given acidosis by $CaCl_2$ administration to increase the numerator. The ammonia N in the urine was thereby brought equal to the urea N. The clearance calculated from the urea excretion alone fell to 50 per cent normal, but calculation from the Urea + NH_3 excretion gave the usual 100 per cent value.

A possible explanation for this behavior is that the urea removed from the blood is in part turned into ammonia in the kidney, and thence excreted as the urinary ammonia. In this case the output of urea plus ammonia nitrogen would represent, more accurately than the urea output alone, the amount of urea removed from the blood by the kidneys. Consistent with such an explanation is Nash and Benedict's (1921) proof that the ammonia of the urine is formed in the kidneys: the probable source of its formation is at least in part the urea removed from the blood.

Regardless of the explanation, the results quoted above indicate that the urea clearance calculated by taking as U the concentration of urinary nitrogen in the form of urea + ammonia is more consistent than the clearance calculated from the urea output alone.

Hence, when the urinary urea is determined by hydrolyzing with heat or urease and estimating the ammonia formed, it is actually somewhat more accurate, for clearance measurements, *not* to remove the preformed ammonia, but to measure it with the ammonia formed from the urea, and use the total urea + ammonia N concentration as U in calculating the clearance.

In calculating the resulting clearance in percentage of normal average, one uses for Equations 1 and 2 coefficients 5 per cent less, viz., 1.26 and 1.76 in place of 1.33 and 1.85.

Urea Clearance Calculated from Excretion of Nitrogen Determinable by Hypobromite

Under the conditions used for the rapid hypobromite determination of urea and ammonia with the manometric apparatus of Van Slyke and Neill, nitrogen gas equivalent to 95 per cent of both the urea and the ammonia is evolved. The N_2 from other urinary substances is slight. One can therefore use the hypobromite method to obtain a close measure of the urea + ammonia excretion. In calculating the results in percentage of normal clearance the fact that the nitrogen yield is 5 per cent below theory just compensates for the average increase of 5 per cent due to addition of the ammonia to the urea. Consequently the same normal standards hold

as for clearances based on excretion of urea alone. Equations 1 and 2, with the coefficients 1.33 and 1.85 unchanged, apply to calculation of results when U represents the total nitrogen determinable with hypobromite in the urine under the conditions specified by Van Slyke (1929). In the urine analysis the only necessary change from the original hypobromite urea method (Van Slyke [1929]) is to simplify it by omitting the preliminary treatment with permutit to remove ammonia.

Procedure for Determination of the Urea Clearance

The data required are analyses of blood and urine, to give the B and U values, and measurement of the urine volume excreted during a measured time interval, so that V in cc. per minute can be calculated.

The following technique has proven practical for hospital routine:

Preparation of the Subject. Except in fairly advanced cases of nephritis, it ordinarily makes no significant difference whether the subject lies down during the test or walks about. Van Slyke, Alving and Rose (1932) found that it made no difference in any subject examined by them who showed more than 50 per cent of normal clearance, but that 3 out of 12 nephritics with less than 50 per cent normal function showed lower clearances if they were up and about. It is therefore desirable that nephritics, who have advanced to less than 50 per cent of normal clearance, rest in a reclining posture during the test, but in other subjects it is not essential.

It is desirable to promote a fairly free flow of urine during the test, in order to diminish the relative error caused by retention of urine in the collecting tubules and bladder at the voidings. For this purpose we routinely give two glasses of water, one at the beginning of the test, and another an hour later, after the first specimen of urine has been collected. This is the only special preparation of the subject which we routinely follow. It may be omitted when for any reason it is desirable to do so.

The gain in constancy of results caused by increasing the urine volume is indicated by the following results. Moller, McIntosh and Van Slyke (1928-a) found that the mean probable deviation, in a given subject, from the average clearance of that subject, was $\pm$ 9.2 per cent when the urine volume was under 2 cc. per minute, and $\pm$ 7.0 per cent when the volume exceeded 2 cc. Addis (1922) obtained routinely a great diuresis by giving one liter of water, with 15 grams of urea, 3 hours before the test, and two glasses (about 400 cc.) each hour thereafter until the test was completed. Under these conditions the mean probable deviation of the clearance in an individual was reduced to $\pm$ 3.7 per cent.

Some of the earlier studies of urea excretion indicated that coffee affected

the results (Addis and Drury (1923) and that clearances taken in the morning might be more consistent than those taken at other times of the day. Page (1933) has found, however, that the amount of caffeine in an ordinary cup of coffee has no measurable effect on the clearance. And general experience in this hospital has shown that the time of day also has no significant effect. The clearance may therefore be taken whenever it is most convenient, and it is not necessary to forbid the taking of a single cup of coffee or of ordinary amounts of food before the test.

Addis and Watanabe (1916) and Addis (1922) gave 15 grams of urea routinely, except in cases with blood urea already high, with the idea that the increased load of urea would put the kidney under a strain and reveal functional deficits that might otherwise be missed. Fowweather (1934) also recommends urea administration on the basis of his experience, but for exactly the opposite reason; giving the urea appeared to prevent an occasional erroneously low clearance which was encountered when urea was not given. Cope (1934), apparently influenced by Fowweather's results, advises the giving of urea. Moller, McIntosh and Van Slyke (1928 *a* and *b*), however, found that the urea administration had no influence, either in raising or lowering the clearance, either in normal or in nephritic subjects. Later, Van Slyke, Alving and Rose (1932) again tested the question carefully, and likewise concluded that urea administration was without effect on the clearance values. It is the writers' belief that the administration of urea increases the accuracy of the clearance determinations only when, by increasing the blood urea content (Fowweather found an average increase of 16 mg. urea N per 100 cc. blood after giving 15 gm. of urea), it augments the accuracy of the blood urea determinations. When a technique for blood urea is used which gives satisfactorily accurate results with the concentrations of blood urea naturally present, there appears to be nothing gained by urea administration. It complicates the procedure, and the methods for rapid blood analysis now available are sufficiently accurate to make the addition unnecessary. One of the advantages of the urea clearance as a renal test is that it is not necessary to complicate it by administration of anything.

Collecting the Urine. It is desirable to collect two specimens of urine, each over a measured period, for which about an hour is a satisfactory time. A single specimen of blood for analysis drawn near the middle of the two-hour interval serves for comparison with both urine specimens. In consequence, two clearances are measured with only one drawing of blood.

To start the first period, the subject, after drinking his first glass of water, empties his bladder completely, without saving the specimen, and a stop-watch is started. At the end of approximately an hour the subject again voids completely and the specimen is saved (Period 1). At the moment when the subject finishes voiding, the time on the stop-watch is recorded as the first period, and the watch is immediately re-started for the second period. The subject then drinks his second glass of water.

At approximately the end of the second hour he again voids completely, the time of this period is accurately recorded, and the specimen is saved (Period 2).

It is not necessary that the time of each period shall be exactly

an hour; it may without harm be longer, up to several hours; or it may be shorter than an hour, provided enough urine (preferably over 50 cc.) is voided to prevent undue error from the volume retained. The essential things are, that the time be exactly recorded, that the approximately complete urine content of the bladder be obtained, and that the blood urea determined represent approximately the average for the period.

In some patients it is impossible to obtain urination at will. In such cases one may continue the collection period for several hours until spontaneous urination occurs, provided the time is accurately recorded. Especially in young children, it is frequently necessary to wait for spontaneous urination. For these the automatic recording device which will shortly be described by Farr (1935) is a great advantage.

Urine Measurement. The urine from each period is measured within 1 per cent. Accurate graduate cylinders of 25, 50, 100 and 200 cc. are kept at hand, and the smallest is used which will contain the specimen.

Collecting Blood. At about the end of the first hour, of the usual 2-hour test, a sample of blood is drawn for analysis. Ordinarily, when no urea has been administered, the change of blood urea content is slow, so that a matter of 10 or 15 minutes time, or even more, sooner or later, is not important. We usually draw 2 cc. or more of blood from a vein into a syringe, thereby obtaining sufficient for duplicates by the usual gasometric methods. When, however, economy of blood is desirable, 0.2 cc. is taken from a finger or ear, or, for small children, from the heel as described by Drucker and Cullen (1925), and the urea is determined by micro-analysis.

Urine Analysis. The rapid hypobromite urea method with the manometric apparatus is used (Van Slyke [1929], Peters and Van Slyke, 1932, p. 379). The *treatment with permutit to remove ammonia,* included in the original description of the method, *is omitted* for reasons already mentioned. The urine is merely diluted 10 or 20-fold, a sample is measured into the manometric chamber, hypobromite is added, the chamber is shaken 1.5, 2 or 3 minutes at 25°, 20°, or 15°, respectively, and the analysis is finished by taking the reading. The improved hypobromite reagent of Van Slyke and Kugel (1933) is used.

Analysis of Blood. The manometric hypobromite method is also routinely used for blood urea. It is applied, as described by Van Slyke and Kugel (1933), to the blood filtrate obtained by deproteinization with zinc hydroxide according to the procedure of Somogyi (1930). The correction of 1.2 mg., recommended by Van Slyke and Kugel for N_2 evolved from non-urea substances in the

Somogyi blood filtrate, is applied. As found by Van Slyke and Kugel, the maximum error by this method is 0.5 mg. of urea nitrogen per 100 cc. of blood. The method is simpler with regard to reagents and procedure than the urease methods, and is as accurate as the urease procedure recommended for routine analyses ("Procedure A, for Determinations in a Series of Blood Filtrates," p. 703, Van Slyke [1927]).

For micro-analyses of blood drawn from skin puncture, the microgasometric urease method (Van Slyke [1927]) has until lately been employed. However, a micro-hypobromite modification has been developed by Farr (1935).

Calculation. It is first necessary to calculate V, by dividing the observed number of cc. of urine excreted during each period by the duration of the collection period in minutes. E.g., if in a period of 62 minutes 155 cc. of urine are collected, $V = 155/62 = 2.50$ cc. per minute.

In case the subject is a child, or an adult differing greatly from ordinary stature, a *correction for body size* is applied to V by estimating the value which V would have for a person of mean adult size. The subject's height is measured in meters, and the necessary correction factor is obtained in Fig. 2 from the proper scale marked 1.73/A. E.g., if the subject cited above were a child with a height of 1.5 meters, the correction factor, in the scale for ages 2 to 15, would be read off as 1.33. The *corrected V value* would then be $2.5 \times 1.33 = 3.33$ cc. For adults the correction for body size may ordinarily be neglected.

The ratio U/B is now calculated by simple division of the result of the urine analysis by that of the blood analysis.

Finally, on either Fig. 3 or Fig. 4, according to the V value (the *corrected* V value in the case of a child), a straight-edge is laid cutting the V and U/B scales at the points corresponding to these values. The clearance value is read off where the straight-edge cuts the center scale. For example, if in the clearance determination cited above for V, U was observed at 250 mg. of nitrogen per 100 cc. and B at 10 mg., the straight-edge would be laid from the point 25 on the U/B scale to the point 3.33 on the V scale of Fig. 3, and would cut the center scale at the point indicating 111 per cent of average normal clearance.

As an alternative to using the line charts of Figs. 3 and 4, one may calculate the results by means of Equation 1 or Equation 2. If one is skilled with a slide rule, this procedure is about as rapid as the use of the line charts. For most analysts, however, the likelihood of error will probably be less in use of the charts.

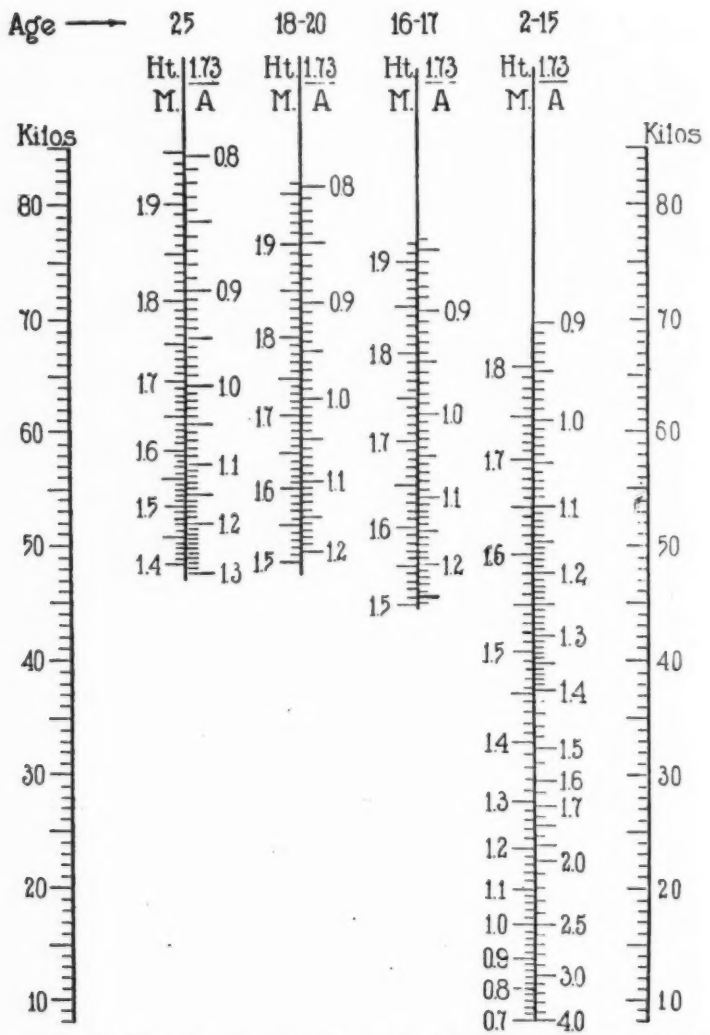

Fig. 2. Chart for correction factors for body size. On each scale the height in meters is on the left and the correction factor 1.73/A is on the right. *A* represents surface area in square meters, 1.73 square meters being taken as standard average for adults. The kilo scales at the right and left indicate normal weights for the heights indicated on the other 4 scales. The kilo scales are added for convenience, and are not used in calculating the clearance corrections.

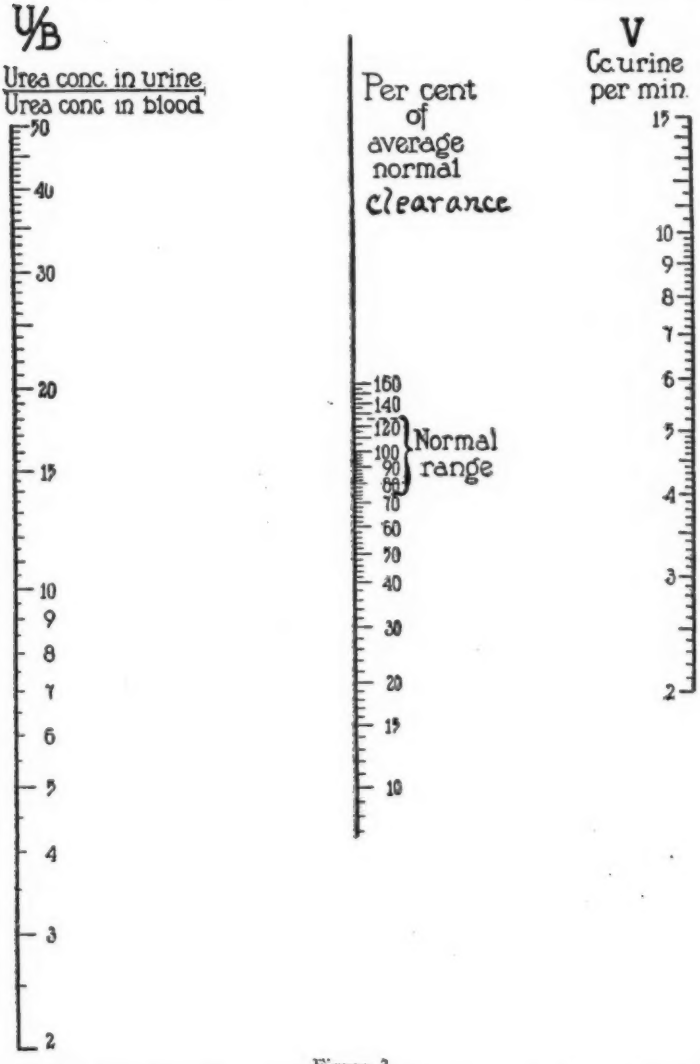

Figure 3

Fig. 3. Line chart for calculating clearances when urine volume (corrected in case of children for body size) exceeds 2 cc. per minute.

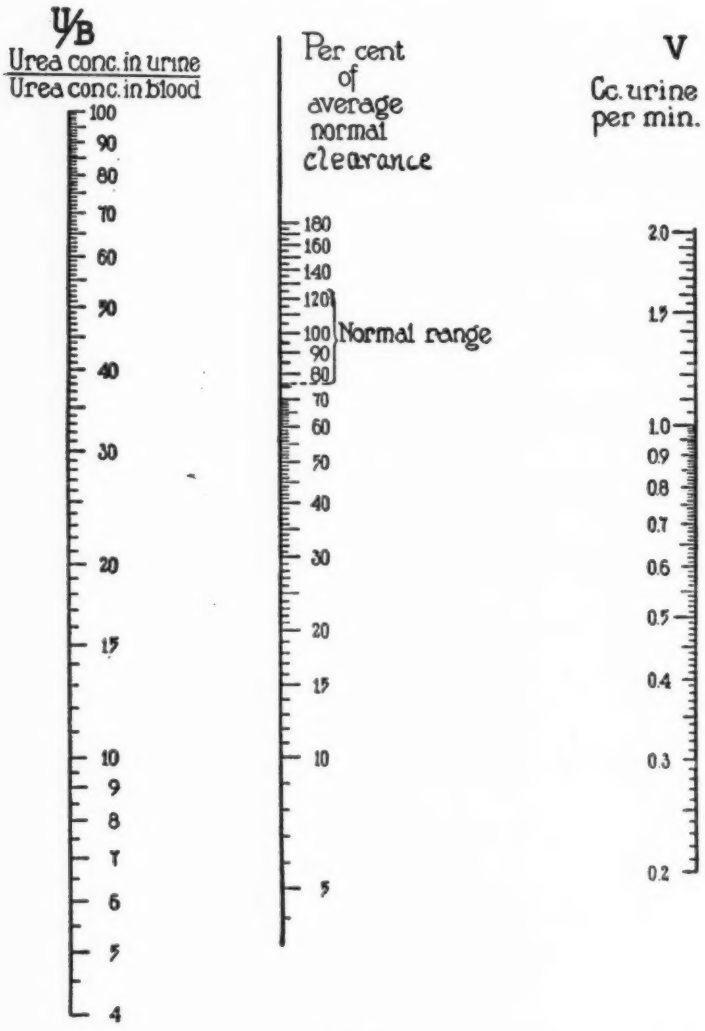

Figure 4

Fig. 4. Line chart for calculating clearances when urine volume (corrected in case of children for body size) is less than 2 cc. per minute.

Clearance by Direct Colorimetric Comparison of Blood and Urine Urea Contents

The technique for this procedure has been described by Van Slyke and Cope (1932). Preparation of the subject and collection of blood and urine are the same as outlined above. Only the procedure for analysis is different. It is convenient where laboratory facilities make colorimetry more practicable than manometric analysis. Because the reverse is the case in this laboratory, the colorimetric procedure has not been used here routinely. However, it has given satisfactory service in the clinic of one of the writer's colleagues (Prof. L. H. Newburgh, of Ann Arbor).

In the light of the results already discussed concerning the desirability of basing the clearance calculation on the ammonia plus urea excretion, the colorimetric clearance may be simplified by omitting the preliminary treatment of the urine with permutit to remove ammonia. The urine dilutions should then be made to 1.05 times the volumes indicated by the graph of Van Slyke and Cope.

Determination of the U/B Ratio as a Substitute for the Clearance

In case it is difficult to obtain urine specimens collected during reliably measured periods for the clearance, one may, in place of the latter, determine simply the *U/B* ratio under conditions which assure a fairly low volume of urine. These conditions are usually obtained by giving a diet with little or no fluids for some hours before the urine sample is taken. Under these conditions *U/B* ratio is normally about 50 or over, and its decrease in nephritis parallels fairly closely the decrease in the urea clearance.

In case there is doubt concerning the ability of the subject to empty the bladder, or concerning the accuracy with which the urine periods are timed, it is better to depend on a *U/B* ratio than an uncertain clearance.

Normal Variation of the Clearance

As mentioned previously, physiological experiments have shown that in normal animals the urea clearance varies parallel with the renal blood flow, which undergoes spontaneous changes from moment to moment. Consequently variation of the clearance over a certain range is an entirely normal phenomenon, is in fact an *evidence of normal functional elasticity of the kidneys.* The usual range in man is approximately from 75 to 130 per cent of *average* normal, as indicated on Figs. 3 and 4. Occasionally a single clearance determination on a normal subject will fall outside this range.

As the clearance decreases in a nephritic case, the variability becomes less; e.g., when the mean clearance is reduced to 10 per cent of average normal, the fluctuations are likely to be limited to between 8 and 12.

Changes in Clearance Caused by Influences of Extrarenal Origin

Because the kidney is not an autonomous organ, but depends on the general circulation for its blood and oxygen supply, and is subject to stimuli of nervous and humoral origin, renal activity, and with it the clearance, is subject to variations of extrarenal origin. These, under exceptional conditions, may become so great as to exceed the usual normal range. The causative conditions are not likely to be confused with those of genuine renal damage, but it is necessary to be on one's guard in order to avoid such error.

As mentioned before, the clearance has been found to vary parallel with the renal circulation. The latter presumably becomes retarded when, with cardiac decompensation, the general circulation is slowed. Consequently, low clearances are encountered frequently in cardiac decompensation, although they are not likely to fall below 20 per cent of normal unless there is a marked degree of specific renal damage in addition to the retarded circulation.

As recently pointed out by Arthur Fishberg, conditions of shock or depression in which the blood is withdrawn from peripheral circulation may depress renal function almost to zero, presumably because the renal blood flow is decreased with the peripheral circulation. Such conditions occur in traumatic shock, gastro-intestinal obstruction, Asiatic cholera, sometimes in acute infections of children, and in various other conditions not accompanied by urinary signs of nephritis. The condition, if it is not permitted to continue till fatal uremia develops, is temporary, and renal function is restored as soon as the circulation recovers. The causative conditions are frequently accompanied by decreased blood volume, and saline infusions will often restore peripheral circulation and renal function.

In surgery the condition of reflex anuria, of nervous origin, is believed to occur. Ordinarily, however, the extrarenal nervous influences appear to play a relatively small part in causing the spontaneous variations in the clearance. Van Slyke, Rhoads, Hiller and Alving (1934) in experiments on dogs, and Page and Heuer (1934) in observations on patients before and after renal denervation, could find no effect of denervation on either the normal level or the normal variability of the clearance.

Back pressure due to partial ureteral, prostatic or urethral ob-

struction may markedly diminish the clearance. Hence, the interpretation of a low clearance as evidence of renal damage is to be made with caution when such obstruction may be present.

The Significance of Clearance Variations in Nephritis

The behavior of the clearance in the various stages and types of nephritis has been observed over periods of years in this hospital (Van Slyke, Stillman, Moller, *et al.*, [1931]), with results which may in part be summarized as follows:

In *acute nephritis* the clearance may remain normal, or it may fall to a tenth of its normal value. Such a fall does not exclude the likelihood of recovery. The necessary condition for a good prognosis is that, if the clearance has fallen, it shall *begin to rise within four months after the onset of the nephritis.* The course of recovery of an acute case in which the onset had been accompanied by a marked drop in the clearance, is shown in the left hand chart of Fig. 5. Complete return to normal clearance may take 8 or 10 months in such a case. It is the beginning of the upturn that indicates the prognosis. Unless this upturn starts within 4 months it is almost certain that the nephritis will become chronic. It may despite the upturn, but is not likely to.

In some cases with acute or degenerative Bright's disease, there is a stage during which the clearance is hypernormal. It may for a time range over 150 per cent of average normal. The phenomenon usually is a stage in recovery. Whether it is due to accelerated renal blood flow or to some other factor in the excretory mechanism is uncertain.

In *chronic nephritis* the fall in the clearance appears to be proportional to glomerular destruction. The condition is irreversible, but a surprising amount of renal destruction, as indicated by the clearance, is compatible with nearly normal activity, provided a good state of nutrition can be maintained, with plasma proteins high enough (over 5 per cent) to avoid edema. We have seen men remain at their work until the clearance had fallen below 10 per cent of normal. When it falls below 5 per cent, however, uremia is imminent, e.g., the terminal case in Fig. 5. These relations of the clearance to the progress of chronic renal disease hold, whether the disease is of the type called hemorrhagic (glomerular), nephrotic, or sclerotic.

A normal clearance does not necessarily indicate absence of pathological changes in the kidneys. As mentioned above, acute cases may run their course, with temporary hematuria, albuminuria and other obvious signs of nephritis without a fall in clearance.

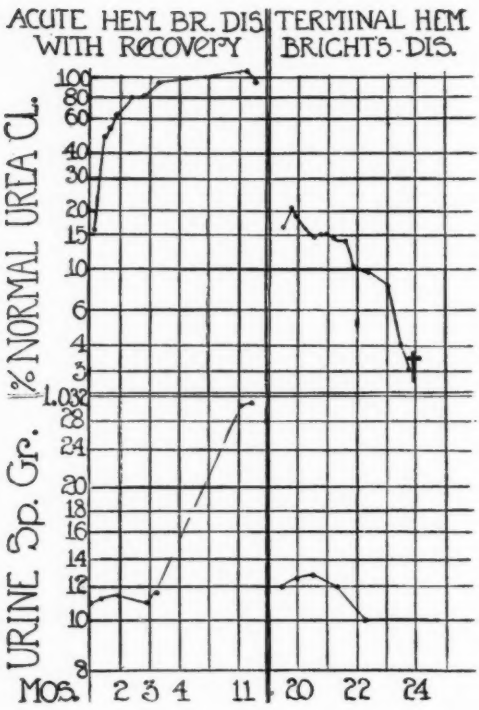

Figure 5

Fig. 5. The upper curves give the urea clearances observed over periods of several months in each of two cases of nephritis. The lower curves indicate specific gravity of urine collected during the last 12 hours of a 24-hour dry diet period, in connection with the Addis specific gravity and sediment tests. The figures across the bottom of the chart indicate months after apparent onset of the nephritis.

Presumably in such cases a normal blood flow through the kidneys is maintained. Again, after partial recovery of an acute case which has suffered a fall in clearance, the clearance may return to normal while albuminuria, hematuria, lowered concentrating power or hypertension persists. Usually such a case goes on to complete recovery, but we have seen one persisting for 5 years. A still less satisfactory outcome is seen in Fig. 6, which shows a case that after

an acute onset and steady climb towards apparent recovery began 6 months after the onset a steady decline ending in uremia after 2 years. Fortunately such a case is the exception.

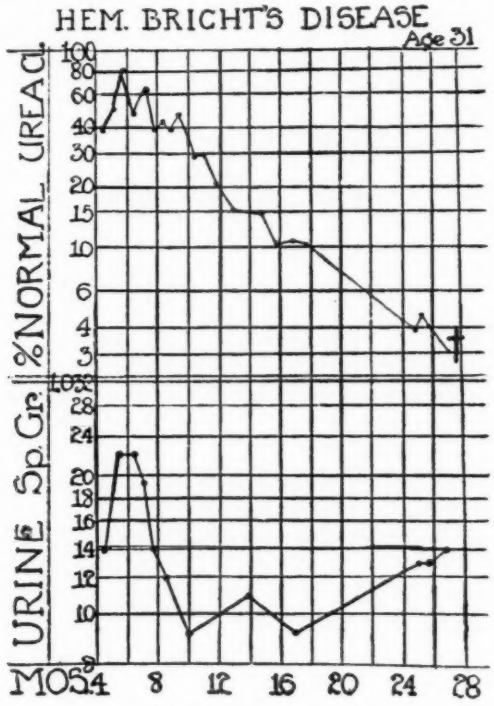

Figure 6

Fig. 6. Clearance changes in a case of nephritis which during the first 6 months promised recovery, but then developed into the progressive chronic form. Significance of curves and figures same as in Fig. 5.

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BACTERIOPHAGE

By FRANK J. SCALERA, L.T.

Newark, N. J.

In 1896, Hankin reported that the filtered waters of the Jumna and Ganges Rivers in India had a definite bacteriocidal action. Since this antiseptic principle was destroyed by boiling, he concluded that it was a volatile substance. However, there is good reason to believe that this man was dealing with bacteriophage.

In 1915, Twort in attempts to culture the virus of vaccinia observed that certain colonies underwent a degeneration and were transformed into a transparent vitreous substance. A transplant of this substance to a normal culture of the same organism would repeat this effect. Passage through porcelain filters did not alter its activity, or diminish its potency, even when the filtrates were diluted 1 to 1,000,000 parts.

In 1917, d'Herelle made a similar observation when he noticed in the stools of patients convalescent from dysentery, a substance which produced complete lysis of young cultures of Shiga bacilli. He called this substance bacteriophage, literally meaning "to eat bacteria."

As the preparation and titration of phage will help us to visualize the experiments which followed, the technique will be discussed at this point. The technique which is now employed at the N. Y. Post Graduate Hospital is as follows:

The media used is plain nutrient beef adjusted to pH 7.4-7.8. Two pure cultures of each organism isolated from the case are prepared on agar slants. One culture is kept in the ice box while the phage is being prepared, should the other become contaminated. A suspension of organisms from the slant is made in broth. Three tubes, each containing 10 cc. of broth are set up. To all three of these tubes is added 0.1 cc. of a light suspension of organisms. To two of the tubes is added 0.50 cc. of a mixed stock phage of a similar organism. The third tube, containing no phage, serves as a control. This set up, labeled 1st generation, is shaken and incubated at 32-37 degrees Centigrade. At the end of four or five hours there should be a good growth in the control tube. Readings of phage are made as zero to 4 plus by comparing cloudiness of the two tubes with the control. One plus and two plus may have sediment at the bottom, three plus and four plus have no such sediment. Whether there is lysis or not, one of the tubes is put through a Berkfeld filter, the

other re-incubated for 24-hour reading. The following day a 2nd generation is similarly set up, using the four-hour filtrate instead of the stock phage and using a fresh 24-hour slant culture of organisms made the day before. All filtrates should be tested for sterility. By successive generations and filtrations, the potency of a phage may be increased. The aim is to obtain a four plus lysis at 24 hours incubation.

Four-hour filtrates of second generation are used for therapy. For intravenous therapy and direct injections into boils and carbuncles, phage prepared in asparagin media is essential. The preparation of such phage is a difficult but not discouraging procedure. As asparagin is not an optimum media for organisms, phage prepared in such media should not be carried beyond the third generation. A weak phage results upon so doing. Lysis should occur in from 8 to 10 hours. Because of the uncertainty of results, the asparagin bacteriophage is always titrated according to the fluid method of d'Herelle. Its titre should not be more than one degree less than the original broth phage used.

Titration of Bacteriophage

Two tubes containing 29 cc. of broth and 1 cc. of suspension of organisms are prepared. A series of 12 tubes is set up. To each tube is added 4.5 cc. of this mixture. To the first tube is added 0.5 cc. of phage to be titred. Five-tenths cc. of this first mixture is carried over to the second tube and, changing pipette each time, this process is carried through the eleventh tube. The twelfth tube containing no phage serves as a control. Readings are made at 4, 24, and 48 hours. The last tube showing lysis is the end point. If this tube is the seventh one, the titer of the phage is read 10^{-7}.

Quantitative studies in the potency of phage have established that a dilution of 10^{-8} of a lytic principle is the smallest amount which is able to produce an appreciable lysis and if larger volumes of phage are used, a dilution still greater than 10^{-8} is able to produce lysis. Dr. Schwartzman, however, summarizes that small amounts of very highly diluted phage can produce lysis as well as large amounts provided that the ratio of the access of oxygen to the fluids used does not exceed 0.5 degrees of anaerobisis, (surface area over total volume). Dr. Schwartzman also concludes that at certain H-ion concentrations both aerobic and anaerobic cultures can be made resistant in the absence of a lytic principle. If the lytic principle is added to these resistant strains, the cultures are able to regenerate it only to a certain extent; however, they do not undergo any visible lysis themselves. The principles regenerated by the resistant cultures are identical in action with each other as well as

with the stock lytic principle. By exposing a lytic principle to the action of buffer mixtures of certain H-ion concentrations its potency is partially inactivated. This partially inactivated lytic principle contrary to the findings of other workers, induces the generation of a principle of maximum activity.

Phenomenon of Bacteriophage

From confirmed observations of both d'Herelle and Brofenbrenner, the interpretation of the phenomenon of bacteriophage remains open for discussion. The conflicting theories can be grouped under two headings.

I. Bacteriophage as being of an exogenous nature; that is coming to the organism from without.

II. Bacteriophage as being of an endogenous nature; that is, as a product of bacteria. Its endogenous nature can be further subdivided into two groups.

(a) Endogenous (enzymic) bacteriophage as an agent which speeds up bacterial fermentation causing the breaking down of bacterial protein.

(b) Endogenous (metabolic) bacteriophage as a product of the life process of bacteria, which kills the organisms themselves, just as man would succumb to his own secretions if provisions were not made for their removal.

Under the exogenous category fall the major contributions of d'Herelle and his followers and under the endogenous category fall those of Brofenbrenner and his followers. d'Herelle's series of thoughts will be outlined, each followed by Brofenbrenner's refutations:

Bacteriophage is a living parasite.

Bacteriophage does not respire. The main constituent of living matter is protein and phage contains exceedingly little if any protein.

It multiplies and is particulate.

The theory of multiplication is not on a clear basis. However, phage is quantitatively dependent upon the multiplication of bacteria with which it is associated and that its being particulate depends upon the ratio of both phage and organisms on the different portions of the media which carry it.

It secretes an enzyme which like other enzymes can be precipitated by 10 volumes of alcohol.

Precipitation is not complete. Some of the lytic agents escape, causing lysis in further generations but d'Herelle claims this lysis

occurs in only one following generation.

It has the power of adaptation. One phage can adapt itself to different strains of bacteria. Its universality is established by complement fixation tests.

It is specific. Lysis of other bacteria are due to original mixed phages. Complement fixation tests prove that different phages do not yield similar fixation reactions.

It can acquire a tolerance for poisons.

This is ingenious but not conclusive. From the field of colloidal chemistry, which deals with non-living matter, analogous experiments have been cited.

We know the following facts concerning bacteriophage to be true:

It stimulates the production of bacteria causing their more rapid growth. During multiplication, phage increases in concentration. Only after a certain time does lysis make its appearance. Following lysis, phage ceases to increase in amount. The bacterial elements swell, take stain less regularly, show granular disintegration and disappear. This process takes four hours.

These facts lead us to believe the following phenomenon:

When bacteriophage comes in contact with a susceptible organism, the growth of this organism is increased. This increases metabolism of bacteria. Their enzymes become more active. Some of the intracellular bacterial elements are digested by these enzymes. This causes osmotic pressure of the bacterial cells. The cells imbibe water and burst.

Streptococcus Bacteriophage

In methods ordinarily employed, the lytic principle was adapted to the organism. In contrast to this method, Dr. Schwartzman, adapting the organism to the lytic principle by training the organism in Clark and Clark's activated sludge phage, was able to isolate a phage specific for a great majority of erysipelas strains. It was also shown by the same worker that streptococcus cultures treated with lytic principles were made inagglutinable by their homologous normal cultures seras, a phenomenon which was known to exist with phage of the coli-typhoid-dysentery group. This phage was also capable of inducing changes of antigenic properties of bacteria. Time and space do not permit his experiments in detail. However, at this point, it is interesting to note that the area of erythema produced by vaccine in 24 hours in one of our patients was reduced to less than half its size when the skin was treated with phage made from the same organisms with which the vaccine was prepared.

There is no doubt that pathogenic organisms are best isolated by plating specimens directly in the patient's own defibrinated blood. As phage susceptible organisms were easily obtained in this way when other cultural methods failed, it was thought advisable to extend the method to organisms of streptococci. Hemolytic and green-producing streptococci were so isolated from a dozen stool specimens. Attempts to lyse these strains with filtrates of their homologous stool specimens were futile.

Drs. Moore and Love recently published favorable results treating cases with bronchial disturbances using phage via the bronchoscope. The phage that was used was more or less a stock preparation.

An effort was made to obtain a specific lytic principle from a dozen sputa from which hemolytic and green-producing streptococci were isolated by the above method, but these cultures showed no lysis with their homologous sputum filtrates.

Hemolytic and green-producing streptococci were so isolated from 20 colonic irrigation specimens. Attempts to lyse these strains with filtrates of their homologous cultures which were left in the incubator for four days were also futile. Piorkowski reports lysing a culture with such a filtrate.

The results were discouraging, but expected as streptococcus bacteriophage is still in its infancy.

THE A. S. C. L. T. QUESTIONNAIRE

4. BIOCHEMISTRY, EXCRETA, AND BODY FLUIDS

By SISTER M. JOAN OF ARC WILSON, R.S.M., M.T., and CHARLES E. BRAMBEL, Ph.D.

From the Department of Laboratories, Mercy Hospital, and the Department of Zoology, Johns Hopkins University, Baltimore, Md.

Data from several departments of the clinical laboratory are here combined into one resumé because of their close association, i.e., biochemistry, excreta, and body fluids. Our first thought was to classify all the information according to bed capacity of the institutions so that the relationship between the different groups would be readily apparent with respect to the diverse methods used for any given determination as well as the variety of determinations utilized by a specific group of institutions, i.e., of a certain bed capacity. But the limited time available in proportion to the voluminous data received prevented the culmination of such a project. We are, however, able to present two charts prepared according to this detailed plan. A study of these reveal some interesting facts and it is hoped that the information obtained from them will warrant the arrangement of the residual data for the other departments according to a similar pattern at some future time.

Excreta and Body Fluids

The routine chemical determinations on urine with the methods used are tabulated in Figure 1. In each group of institutions according to bed capacity, the predominating methods for albumin and glucose reached a high percentage in favor of the heat and acetic acid test and Benedict's respectively. For acetone, Rothera's method is preferred by the majority in the group of 200-300 beds only, while Lange's predominated in all the others. For bile pigment, Smith's test equaled the same percentage as Cmelin's in the group of 100-200 beds, while the latter was more used in all the other groups. Other methods not so extensively used are indicated in percentage of the total number answering in each group according to bed capacity.

The routine examination with methods utilized on the gastric and duodenal fluids are summarized in Figure 2. The most popular

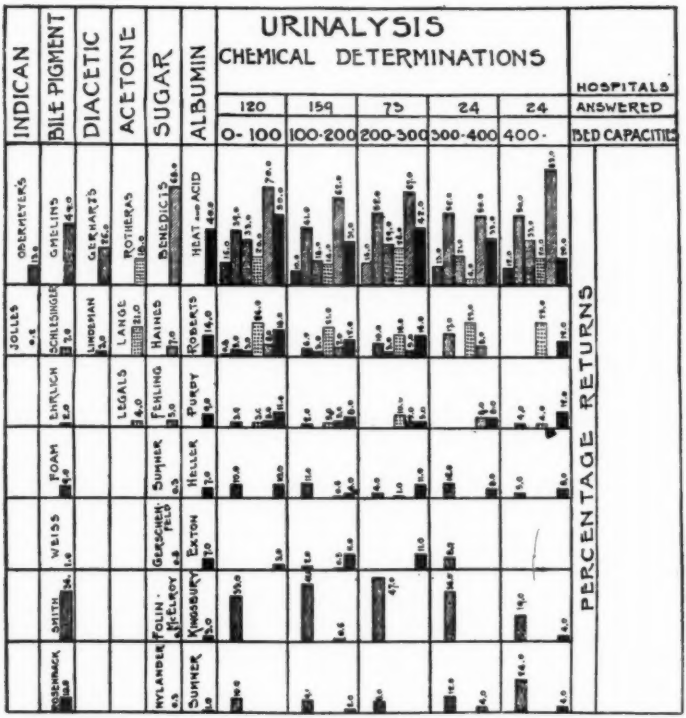


Figure 1

test meal given preliminary to a gastric analysis, is the Ewald, except in the groups of 0-100 beds and 200-300 beds, where the Rehfuss Fractional test takes precedence, and in only the first three groups was this latter procedure reported at all. Very few gave any reports for acidity other than the free HCl and total acidity. The greater number in each group prefer Kelling's method for lactic acid. It was interesting that the Benzidine test for occult blood predominated in every group except that of 300-400 beds, from which the Guaiac test only was reported. The remaining miscellaneous determinations were given by only a very few.

Other methods reported were as follows:

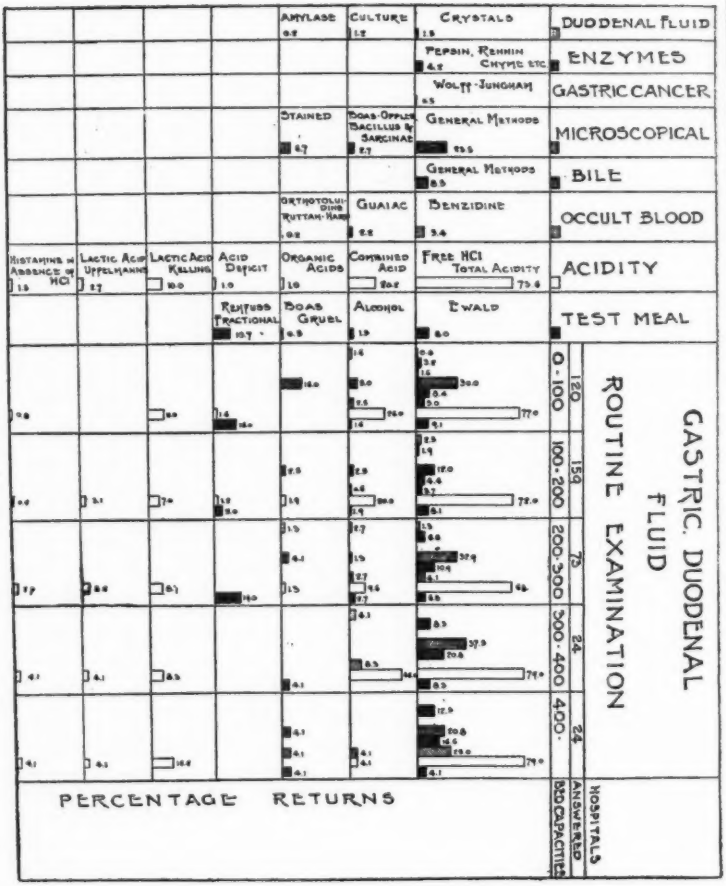

GASTRIC DUODENAL FLUID
ROUTINE EXAMINATION
DUODENAL FLUID
ENZYMES
GASTRIC CANCER
MICROSCOPICAL
BILE
OCCULT BLOOD
ACIDITY
TEST MEAL
Amylase
Culture
Crystals
Pepsin, Rennin Chyme etc.
Wolff-Jungham
Stained
Boas-Oppler Bacillus & Sarcinae
General Methods
Orthotoluidine Ruttan-Hart
Guaiac
Benzidine
Histamine in Absence of HCl
Lactic Acid Uffelmann's
Lactic Acid Kelling
Acid Deficit
Organic Acids
Combined Acid
Free HCl
Total Acidity
Rehfuss Fractional
Boas Gruel
Alcohol
Ewald
0-100
100-200
200-300
300-400
400-
120
154
73
24
24
PERCENTAGE RETURNS
RBC
CAPACITIES
ANSWERED
HOSPITALS

Figure 2

QUANTITATIVE GLUCOSE

Bed Capacity / Methods	Total percent	0-100 according to	100-200 number	200-300 responding	300-400 from each	400- group	Total percent from all
Benedict	87	76	69	9	66		78
Fehling	7	1	7	—	8		5
Folin and Wu	4	6	5	—	4		5
Sumner	—	—	2	4	4		1
Exton	0.8	—	—	4	12		1
Polariscope	2	0.6	—	—	4		1

QUANTITATIVE ALBUMIN

Esbach	56	60	63	62	33	58
Tsuchiya	7	6	5	4	12	7
Folin	1	—	—	—	—	11
Purdy	13	13	7	8	12	12
Exton	4	2	9	12	20	6
Kingsbury-Clark	—	1	1	—	4	1

RENAL FUNCTIONAL

Phenolsulphonphthalein	89	85	89	92	87	87
Mosenthal	22	18	30	25	46	21
Urea Clearance	2	6	8	17	13	6
Concentration, Dilution	5	4	10	—	—	5
Creatinine Clearance	8	—	1	—	4	8
Fishberg	—	0.6	6	—	4	2

CYSTOSCOPIC SPECIMENS

Routine urine analysis	12	11	16	25	16	13
Microscopic only	67	58	67	71	50	63
Stained smear: Gram	2	3	15	4	8	6
Acid fast	10	6	27	8	12	12
Stain not given	14	19	19	16	25	17
Culture	50	41	61	79	54	50
Guinea-pig inoculation	8	13	18	16	8	12
P.S.P.	6	4	9	8	4	6
Cell count	1	1	4	4	8	3
Albumin	5	2	2	—	—	3
Urea	1	2	4	8	—	3

FECES

Bed Capacity / Methods	Total percent	0-100 according to	100-200 number	200-300 responding	300-400 from each	400- group	Total percent from all
Macroscopic-Appearance	23	29	15	37	29		25
Microscopic-Gen. Exam.	51	54	72	78	58		58
Ova and Parasites							
Kofoid and Barber	27	17	22	17	25		22
Shearer	25	4	5	—	4		15
de Rivas	2	6	11	8	8		6
Pepper	—	1	—	—	—		0.5
Stiles	0.8	3	1	—	—		2
Amoeba: Warm stage	62	27	79	83	62		58
Donaldson's I-E	15	20	34	17	20		17
Schaudinn	10	12	20	4	12		12
Thionin or Neut. Red	—	1.7	3	—	—		1.1
Culture	—	—	—	12	8		1

Bed Capacity Methods Total percent	0-100 according	100-200 to number	200-300 responding	300-400 from each	400- group	Total percent from all
Occult Blood						
Benzidine	82	67	83	75	83	76
Guaiac	13	16	27	20	4	17
Both Benz. and Guaiac	8	4	21	17	8	9
Miscellaneous	3.6	3.1	5	—	4	3.9
Bile						
Schmidt	30	42	38	60	29	38
Gmelin	23	27	38	20	29	27
Miscellaneous	10	16	21	20	20	15
TRANSUDATES AND EXUDATES						
Specific Gravity	31	26	50	54	46	35
Albumin	22	22	27	17	42	24
Cell count—total	30	33	43	50	37	35
differential	18	24	16	29	13	20
Stained smear—Gram	55	51	67	50	50	53
Acid fast	3	8	12	13	21	8
Coagulation	7	8	5	—	4	6
Rivalta's Reaction	5	8	8	13	8	7
Culture	56	51	59	75	54	55
Guinea-pig inoculation	7	6	15	—	—	7
Wassermann	1	0.6	1	—	—	1
Colloidal Gold or Benzoin	—	—	—	—	4	0.2
Calcium	—	0.6	—	—	—	0.2
Phosphorus	—	0.6	—	—	—	0.2
Sugar	2	1	—	—	—	1
Tumor and cancer cells	8	1	—	8	—	1
SPINAL FLUID						
Routine: Appearance	4	6	5	12	8	6
Pressure	2	0.6	1	—	—	1
Total cell count	78	79	86	87	79	86
Differential	27	18	19	8	12	17
Bacteriological: Gram	34	37	39	29	12	35
Acid fast	3	6	2	—	8	4
Globulin: Pandy	10	17	20	4	33	16
Ross-Jones	15	10	14	12	16	13
Nonne-Apelt Reaction	4	7	5	4	—	5
Noguchi	5	1	4	4	4	3
Total Protein	—	3	5	—	12	3
Wassermann: Comp. fixation	32	28	5	46	33	34
Kahn	7	12	15	12	16	12
Klein	0.8	0.6	4	—	—	1
Meinicke	—	0.6	4	—	—	0.2
Both Kahn and Comp. fixation	1	0.6	5	25	12	4
Colloidal Gold	27	43	75	79	79	48
Gum Mastic	9	6	14	20	8	9
Colloidal Benzoin	3	1	2	—	—	2
Sugar	48	45	55	50	58	49
Chlorides	1	6	10	4	4	5
Culture	32	37	41	46	16	33
Guinea-pig inoculation	0.8	2	2	4	8	2

URINE NITROGEN ESTIMATIONS

Urea: Marshall	7	6	15	4	4	8
Van Slyke-Cullen Mod.	7	0.6	12	13	13	6
Karr	0.8	0.6	5	4	—	1.7
Meyers	0.8	1	—	—	—	0.75
Keller	0.8	1	3	4	—	2
Leiboff & Kahn	—	0.6	—	4	—	0.05
Folin-Wu, and Mod.	11	4	12	17	25	10
Doremus-Hinds	5	6	7	8	4	6
Benedict	0.8	1	—	—	—	0.75
Creatinine: Folin	19	17	26	33	42	22
Benedict	—	1	4	—	—	2
Ronchese-Malfatti	0.8	—	—	—	—	0.25
Meyers	2	2	3	—	4	2
Van Slyke	—	0.6	—	—	—	0.25
Weyls and Mitchell	—	—	1	—	—	0.25
Uric Acid: Folin	9	6	7	17	21	9
Benedict-Franke	12	11	16	17	25	13
Morris-McLeod	—	0.6	—	—	—	0.25
Cook	—	1	1	—	—	0.75
Meyers	—	1	1	—	—	0.75
Brown	0.8	0.6	—	—	—	0.05
Ammonia: Van Slyke	3	4	5	4	—	4
Ronchese-Malfatti	3	1	—	—	4	2
Folin-Bell, Mod.	6	9	8	4	21	8
Benedict	0.8	2	1	—	—	2
Meyers	—	1	1	—	—	0.75

The following additional determinations were reported by only a very few (from 0.5% to 1.5%):

Urine: pH
Bence-Jones protein
Diazo
Osazone (Phenylhydrazine)
Fermentation
Arsenic
Lead
Mercury
Calcium
Phosphorus
Albumin (Sulphosalicylic Acid)
Melanin

Spinal Fluid: Levinson's Test
Tryptophane

Feces: Gram stain
Fermentation
Calcium
Phosphorus
Lead
Fat

Fluids: Malignant cells
Histamine for achlorhydria

Each of the following was reported by only one institution:

Urine: Urobilinogen, galactose, alkali tolerance, creatine by Folin's micro method, total nitrogen, cell count using urine counting chamber, diastatic unit (Wohlgemuth), amylase, lactase, trypsin, Stowe and Burke method for blood in urine, simultaneous quantitative urine and stool urobilinogen in jaundice and liver functional tests, horismascopic test for albumin, proteose test and preparations, daily routine iodine (Lugol's) in all thyroid patients, routine benzidine

BIO·CHEMISTRY RETURNS

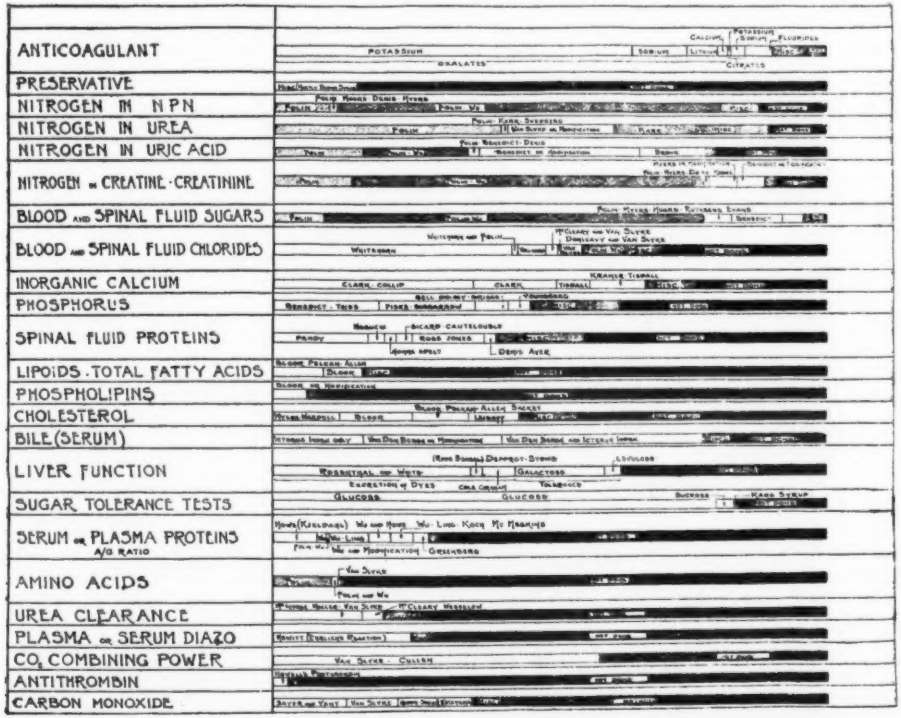


Figure 3

on sediments for hemolized blood, Folin as well as Esbach for albumin, Volhard's and Purdy's chlorides, Ronchese-Malfatti formalin for ammonia, dilution of pentose, alcohol, amino-acids (Folin), sulphates, calcium balance (urine and feces), occasional Mosenthal test for renal insufficiency, McLean Index for kidney function, Gram's iodine and acetic acid for bile (preferred because of simplicity), total sulphur in disturbed sulphur metabolism (valuable in chronic arthritis), bromides, iodides, hematoporphyrin and lactose, creatine run with creatinine in muscular dystrophy, analysis of calculi (for diet regulation in renal diseases), quantitative albumin with potassium ferrocyanide and acetic acid and centrifuged, titration for acidity, and quantitative hormone determination.

Feces: Enzyme determination (diastase and trypsin), blood by Alvairy and Wright method, Kastle, Amos and Benoit phenolphthalein test for blood. calcium balance (feces and urine), pH, amylolytic activity (Wohlgemuth), fat.

Spinal Fluid: Acetic anhydride test for initiative process, calcium and phosphorus (useful chiefly in rare cases for differential diagnosis), alcohol.

Gastric: Washings for tubercle bacilli in suspected cases when sputum is negative—also in children and when sputum is unavailable, followed by guinea-pig inoculation if smear is negative (very valuable); Rice test meal ten P. M. previous evening with special technic and subsequent analyses.

Sputum: Culture of sputum in patient's own blood, whereby the specific pathological organism may be isolated, and vaccine made therefrom; blood not only acts as nutrient material for pathological organisms but it still retains its properties of destroying the other organisms (Cohen's Pathogen-Selective method). Doglio's stain, NaOH applied to unstained sputum for mycelia-wet fungi, lactophenol to unstained sputum for actinomycetes, Greenfield-Corper for tubercle bacilli, asbestosis.

Biochemistry

Figure 3 shows that twenty-two biochemical determinations are made use of by the institutions answering the questionnaire. From this chart a generalized composite hospital routine may be obtained by inspection, i.e., those procedures in clinical chemistry which might be of general diagnostic value. As anticoagulant for the blood, 82.4% use an oxalate with potassium oxalate predominating at 65%, and a very few reported citrates or fluorides. Only 15% use a preservative for the blood and these mainly for blood sugars. The chemical determinations reported are listed in the following tables, in the order of their popularity. Two percentages are given, the one on the left being the total percent reported, while the value on the right refers to the predominating methods only.

	Percentage Use	Determinations	Predominating Methods	Average Percentage
Group I	97	Blood and spinal fluid sugars	Folin, and modifications	84
	87	Nitrogen in: Urea, N.P.N. Creatinine, Uric Acid	Folin, and modifications	60
	85	*Sugar Tolerance Tests	Janney and Isaacson	85
	83	Bile (Serum)	Van den Bergh	66
	76	Inorganic Calcium	Clark or Clark-Collip	51
	70	Blood and spinal fluid chlorides	Whitehorn, and modifications	44
Group II	63	Liver Functional tests	Rosenthal-White (Excretion of dyes)	37
			Schlay & Schloss (Galactose Tolerance)	16
	60	CO_2 combining power	Van Slyke-Cullen	59
	59	Phosphorus	Benedict-Thies	19
			Fiske-Subbarow	18
	56	Spinal fluid proteins	Pandy	17
	55	Cholesterol	Bloor, and modifications	23
	43	Carbon monoxide	Sayer and Yant	12
Group III	30	Plasma or serum proteins	Wu, and modifications	17
	29	Plasma or serum diazo	Hewitt (Ehrlich's Reaction)	25
	23	Urea clearance	McIntosh-Moeller-Van Slyke	12
	22	Total fatty acids	Bloor, and modifications	16
	14	Amino acids	Folin, and modifications	12
	8	Phospholipins	Bloor, and modifications	6
	5	Antithrombin	Howell's Prothrombin	3

*Emphasis was placed upon the fact that the quantity of sugar administered for the glucose tolerance tests was not significant, as seven different amounts were reported, varying from 50 Gm. to 120 Gm., and from 1.5 Gm. to 2 Gm. per kilo weight.

The factors affecting the above distribution of the different procedures into three groups are not entirely clear. One might hazard the suggestion that the minority group consists mostly of specializations, whose importance has not as yet been appreciated sufficiently for their utilization in a large percentage of hospitals, while those in the intermediate group have been recognized as of great diagnostic value by approximately one-half of our total group of institutions. Other factors might include the type of institution, financial status, or laboratory personnel, and facilities.

In Figure 3, the solid black indicates the total percent either not using the procedure or not reporting it; and those in use by a very small percent are included in miscellaneous.

The following additional determinations were reported by only a very few (from 0.5% to 2.5%):

Blood bromides	Cholesterol esters
" pH	Micro blood sugar
" indican	Clotting index
" phosphatase	Alcohol in blood or fluids
" iodine	Double Glucose Tolerance Test
" iron	50 Gm. glucose one-half hour apart
" phenol	Milk analyses for protein, fats, carbo-
" amylase	hydrates, formaldehyde, and bac-
" fibrinogen	teria.

Each of the following was reported by only one institution:

Methemoglobin, Congo Red test for amyloidosis, retractility test, stain detection for blood—also human blood, Jameson's test for cancer, cystine content in finger nails, creatinine clearance, creatine and creatinine ratios in both blood and urine at same time (muscular dystrophies), Schmidt's nuclei test, proteose extraction in blood and urine, anterior pituitary hormone (prolan) in blood and urine, stroke volume of the heart (Keller), KOH content of soaps used in hospital, blood carotin (Connor), oxygen capacity and combining power (Van Slyke), intra-spinal autogenous serum in chorea and some dementia praecox cases, 1-1500 HCl intravenous, vital capacity. Water for residue, alkalinity, sulfates, chloride. Arsenic in cloth, hair, and liver. Lead in dust. Silica in dust, tissues, etc.

NEWS AND ANNOUNCEMENTS

BOARD OF REGISTRY

OF A. S. C. P.

The next meeting of the Board of Registry will be held in connection with the convention of the American Society of Clinical Pathologists, in Kansas City, May 8-10, 1936. The members of the Board now have under consideration various proposals for the classification of technicians, among them more rigid requirements for the title of Medical Technologist for non-college graduates.

The Registry's devotion to the welfare of the technician is exemplified in its warfare against the technician interne evil. Recently a commercial school inserted an advertisement in a hospital journal offering its students to hospitals to serve without pay and for just maintenance.

The Registry promptly countered with a circular letter to all hospitals of the country (about 6000) cautioning them against this practice. This strategic move effectually checked this incipient effort to lower the status of the technician.

The Registry is deluged with routine business and correspondence necessitated by the issuance of new certificates to our more than three thousand registrants. We ask the kind indulgence of our friends to any delay until the present rush is over.

Additional help has been secured. Our personnel now numbers four not counting the chairman, all of whom are working at top speed to keep from falling behind.

Interest in the Registry has had an enormous spurt of late. A wide publicity campaign is under consideration. As a result of the activity of the Registry there has been a marked reduction of incompetent technicians and a greater demand for qualified registered laboratory workers, all of which will redound to their scientific and economic status. Most of the professional employment agencies are now giving preference to registered technicians.

Three hundred and eighty applicants passed the October examination and were admitted to the Registry as of January 1, 1936.

The next semi-annual examination will be held in April. Applicants desiring to enter this class should file their applications immediately.

FOURTH ANNUAL CONVENTION OF THE AMERICAN SOCIETY OF CLINICAL LABORATORY TECHNICIANS

Greater Kansas City and Excelsior Springs, Missouri, host to the American Society of Clinical Laboratory Technicians, meeting May 11 through the 13th, supplements the usual convention program by blending the culture of the East, the vision of the West, the energy of the North, and the hospitality of the South.

Present plans indicate that the annual session, which will be held this year at the Elms Hotel, Excelsior Springs, a suburb of Kansas City, will be of exceptional interest to everyone attending. Those wishing to appear on the program are urged to send their titles of papers to Frieda H. Claussen, M.T., Chairman of the Program Committee, 469 Laurel Avenue, St. Paul, Minnesota, as soon as possible, as there are a few places open on the program for papers presented by technicians.

STATE

Maryland

At their January Meeting, held at Mercy Hospital, the Maryland technicians were privileged to hear Dr. Maxwell H. Wintrobe of Johns Hopkins Medical School, speak on the "Use of the Hematocrit in the Determination of Blood." This very interesting talk was supplemented by instructive lantern slides and also by the demonstration of the hematocrit readings in various blood diseases. Following his talk, Dr. Wintrobe very kindly answered numerous questions relative to his subject, which proved of great help to those present.

Washington

The Inland Empire Society of Clinical Laboratory Technicians, which was organized a year ago with Jacqueline Bahrenburg as president, gave its first annual banquet and installation of officers, January 10th, 1936.

Mary Lowry was installed as president, Helen Douglas as vice-president, and Fay Yenter as secretary and treasurer.

The pathologists of Spokane, who have given so generously of their time and encouragement to the Society, were the guests of honor and contributed to the enjoyment of the occasion.

Foreign

The Nobel prize in Medicine this year has been awarded to Prof. Hans Spemann, ordinarius in zoology, at the University of Freiburg-im-Breisgan, in recognition of his work on what is called the organ producing effect.

BOOK REVIEW

CLINICAL LABORATORY METHODS AND DIAGNOSIS. A textbook on Laboratory Procedures and their Interpretation. By R. B. H. Gradwohl, M.D., Director of the Gradwohl Laboratories, Director of the Gradwohl School of Laboratory Technique, Director of Laboratories, St. Louis County Hospital. Chief of Staff and Pathologist to Christian Hospital. Lt. Commander, Medical Corps, Fleet, U. S. N. R.. C. V. Mosby Co., Publishers, St. Louis, 1935. Pp. 1028, Price $8.50.

Drawing from many sources of literature as well as his own tremendous practical experience, Dr. Gradwohl has compiled a Gargantuan encyclopedia of laboratory methods and their interpretations in the diagnosis and prognosis of disease. Few procedures ever in use at any time in the clinical laboratory have been omitted.

There are eighteen chapters in all. They cover General Considerations, Urinalysis, Blood Chemistry, Hematology, Gastric Analysis, Examination of Puncture Fluids, Examination of Sputum, Special Tests, Feces, Parasitology, Bacteriology, Serology, Basal Metabolisms, Postmortem Examinations, Histological Technic, Preparation of Museum Specimens, and Toxicology, followed by a final chapter on the reagents and equipment necessary in the laboratory.

The chapter on blood chemistry is the poorest and most out of date section in the book. It is to be regretted that this chapter comes early as it might discourage the more dilatory student. Here the methods given are pure "rule of thumb," the calculations inexact and the bibliography about 20 years behind the times. Notably there is no mention made for the determination and fractionation of serum proteins.

In the next chapter on hematology, however, the author redeems himself by giving an excellent resume of the more recent work in this field, in particular in his discussions, illustrations and case histories in reference to the Schilling differential blood count. A variety of the more unusual methods for staining blood smears are given.

The book includes several welcome descriptions of laboratory procedures not found in the ordinary text. In particular these include the preparation of antigens for tests for hypersensitiveness, paraffin section methods for centrifugates of puncture fluids, several classifications of yeasts and fungi, rabies, and the preparation of museum specimens. The section on toxicology is valuable and very

complete. We are delighted that the author has attempted a systematic discussion of these subjects. They are conspicuous by their absence from most texts and practical demands are often made on the laboratory with reference to them.

Almost 150 pages are devoted to bacteriology. Following a general discussion of the usual methods of the cultivation and staining of bacteria come methods for the bacteriological diagnosis of specific diseases (conjunctivitis, gonorrhea, etc.). The technician will find here practical and detailed information concerning the testing of disinfectants, preparation of bacteriophage, aseptic cultivation of surgical maggots, and the cultivation of gonococci. The chapter on serology describes the Noguchi and Kolmer modifications of the original Wassermann reaction, the Kahn and Kline and the Hecht-Gradwohl tests (which makes use of the natural complement and amboceptor present in most serum) in the serodiagnosis of syphilis. "Tissue Cutting and Staining" is a complete and very excellent chapter. We are glad to see the original staining methods of Cajal for Central Nervous Tissue are included.

The style as a rule is good and well sustained but the author occasionally lapses into obscurity. For example on page 620, "In sprue, the blood shows a mild secondary anemia with a marked reduction in hemoglobin, the color index is higher than normal—" would appear to be a contradiction of definitions. And on page 159 "Experimental proof that *hyper*glycemia results from hypoendocrine function was obtained by Janney and Isaacson in the case of the thyroid where *hypo*glycemia regularly developed after thyroidectomy" seems rather obscure. An irritating trick of Dr. Gradwohl's is his habit of quoting profusely from many sources. It is not at all unusual to find several pages of quotation from original articles with the method transposed in toto. At times this gets tiresome, and the reader would prefer the material in semi-digested form at least, with the author's opinion of his results with the particular method under discussion.

These minor shortcomings are, however, readily overlooked when the book is viewed in its entirety. The author is to be congratulated upon his meticulous attention to detail, the breadth of scope of the work and the care and application he has used in assembling his wealth of material.

ABSTRACTS

KAHN READING: MODIFICATION OF HECHT'S SUDAN METHOD; STAIN TECHNOLOGY, Vol. 10:4, October, 1935.

Add a knifetip portion of Sudan R 111 (I. G. Farben) to 100 cc of Kahn's extract; filter after twenty-five hours. According to the titer, add the required amount of saline and incubate ten minutes at room temperature. Pipet the extract into the serum, shake five minutes, add 0.5 cc saline to each tube and centrifuge ten minutes at 2000 r.p.m. A dot of red precipitate, after decantation, indicates a positive reaction.

Original Article: E. Weichherz; Die Kahnsche Reaktion mit Farbung nach Hecht, Cas. lek. ces., 1934, 1096. (Abs. in Zentbl. Bakt. 1 Ref. 117, 438, 1935).

STAINING OF THE PROCESSES (FLAGELLA) OF THE HUMAN ERYTHROCYTES: STAIN TECHNOLOGY, Vol. 10, No. 2, April, 1935.

In this article the following technic is given for staining the processes of erythrocytes; formerly these were observed only in fresh blood preparations:

To 2 to 3 cc of sterile Ringer's solution in a sterile test tube, add 1 mg. of hirudin or some such anticoagulant. Draw up into a Pasteur pipet about 0.5 cc of this liquid and mix it with a drop of fresh normal human blood. Transfer this blood to the test tube and incubated from 40 to 50 minutes at 37° C. Tilt the glass slips at an angle of about 40° and let a small drop of the blood mixture run down from the upper edge. Move carefully to a horizontal position and air dry. Soak film with the following mordant: 10% tannic acid, 50 cc; saturated aqueous $FeSO_4$, 25 cc; saturated alcoholic basic fuchsin, 5 cc. Make up fresh each time and filter at least twice. After covering the slide, warm slightly and let mordant act 20 minutes. Wash in running water and blot dry. Stain 20 minutes with a fresh Ziehl-Neelsen carbol fuchsin (containing 1% fuchsin); wash thoroughly in tap water, blot dry.

Erythrocytes stain light pink, dark red or occasionally purplish black, in most cases irregularly. If there are endoplastic bodies they stain a deeper hue than the surrounding cytoplasm. The extroflecting processes are light pink, like the cell membrane.

BLOOD PLATELET COUNT; OLEF'S METHOD: Jour. Lab. and Clin. Med., Vol. 20:4, January, 1935.

The most accurate procedures for counting the blood platelets are the true indirect methods. Olef's method has been found both accurate and dependable, as it adheres to the leading principles that must be taken into consideration in counting platelets.

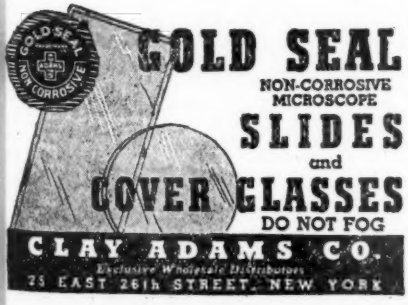
Materials needed: Acid cleaned slides and cover-slips, paraffin cups, and applicators with paraffin coated tips, Olef's platelet solution, and vaseline.

Technic: Cleanse finger tip with green soap and dry with alcohol and ether after warm hand bath. Puncture deeply and after allowing one or more drops to flow, wipe gently, cover puncture with one drop of solution (Olef's); turn hand over and allow blood to flow through solution into cup containing three to four drops of solution. Mix thoroughly and allow to stand a few minutes. Stir well again, and transfer with applictaor to glass slide, place cover-slip over preparation and seal with vaseline. Allow to stand from ten to fifteen minutes.

Olef's Solution (using dye which however, he claims unnecessary):

	Gm. or cc.
Sodium metaphosphate (special-Howe and French)	1.0
Sodium chloride	0.4
Dextrose	0.1
Sodium bicarbonate	0.1
Brilliant cresyl blue	0.15
Distilled water	100.0

In this solution both the platelets and reticulocytes are well stained so that a reticulocyte and thrombocyte count can be done simultaneously. When not in use, the solution should be kept in a cold place and should be filtered every few days.



PAST DISCOVERIES

Edwin Klebs discovered diphtheria bacillus in 1883.

Pasteur vaccinated against anthrax in 1883.

Metchnikoff stated the phagocytic theory of immunity in 1883.

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PIONEERS

Max von Pettenkofer
(1818-1901)

Founder of experimental hygiene. He investigated the metabolism in respiration. He also demonstrated hippuric acid, creatin, and creatinin in the urine. He was a student of public hygiene.